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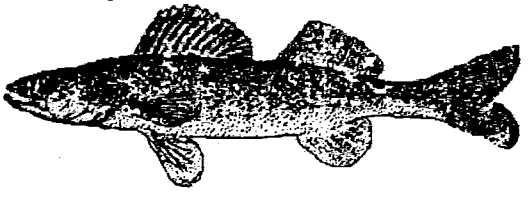
Effects of Exposure to Ammonia on Sensitive Life Stages of Aquatic Organisms

Contract Report 68-01-5832/A

Center for Aquatic Ecology

Keturah A. Reinbold
and
Stephen M. Pescitelli

October 1982
Reprinted January 1990



Aquatic Ecology Technical Report 1990
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Effects of Exposure to Ammonia on Sensitive Life Stages of Aquatic Organisms

by

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Final Report to U.S. Environmental Protection Agency
Region V, Chicago, Illinois
Walter Redmon, Project Officer

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ABSTRACT

Effects of ammonia on early life stages of four fish species (walleye, white sucker, channel catfish, and green sunfish) and throughout the life cycle of *Daphnia magna* were evaluated. *D. magna* was less sensitive to ammonia than were the fish species tested. The lowest concentration of un-ionized ammonia found to cause an adverse effect on daphnids was 1.3 mg/L.

Fish eggs were not affected by exposure to un-ionized ammonia at 0.96 mg/L, the highest concentration tested in this investigation. Concentrations as low as 0.05 and 0.06 mg/L caused a delay in time to swim-up and a significant reduction in growth, respectively, in the fish species tested.

CONTENTS

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I. INTRODUCTION

Ammonia is an important toxicant to fish and other aquatic organisms. It enters natural waters from several sources, including sewage effluents, industrial wastes, and runoff from agricultural feedlots.

Considerable data are available on ammonia toxicity as reviewed in the U.S. Environmental Protection Agency's "Quality criteria for water" (1977) and by Willingham *et al.* (1979). There are, however, important aquatic species for which little or no data on ammonia toxicity are available, and information on ammonia toxicity to sensitive life stages exists for only a few species. Data on effects on sensitive life stages of additional species are needed to evaluate effects of ammonia discharges and to determine levels that allow protection of aquatic life throughout their life cycles.

The objective of this study was to evaluate effects, particularly sublethal effects, of ammonia on early life stages of five aquatic species native to the Midwest for which either little information on ammonia toxicity was available or data on sublethal effects of ammonia or effects on early life stages were lacking. Selected for this investigation were the microcrustacean *Daphnia magna* and four fish species: walleye (*Stizostedion vitreum*), white sucker (*Catostomus commersoni*), channel catfish (*Ictalurus punctatus*), and green sunfish (*Lepomis cyanellus*).

II. CONCLUSIONS

Daphnia magna was less sensitive to ammonia in both acute and sublethal effects tests than were the fish species tested. The lowest concentration of un-ionized ammonia found to cause an adverse effect on the daphnids was 1.3 mg/L, while effects on fish occurred at concentrations as low as 0.05 mg/L.

Fish eggs were not affected by exposure to un-ionized ammonia at 0.96 mg/L, the highest concentration tested in this investigation. Concentrations as low as 0.05 and 0.06 mg/L caused a delay in time to swim-up and a significant reduction in growth, respectively, in the fish species tested.

Walleye egg viability was poor, as was larval survival during the first 2 wk after hatch, when some individuals did not begin to feed. Additional information is needed on techniques for rearing this species before it can be used successfully in tests on early life stages.

III. RECOMMENDATIONS

1. More investigation of laboratory rearing techniques for walleye is needed before this species can be used in tests on early life stages.

2. The test of the effects of ammonia on early life stages of green sunfish should be repeated since a concentration of ammonia causing an effect on growth could not be identified in our tests.

3. Results that should be considered in setting water quality standards are the delay in time to swim-up at 0.05 mg/L un-ionized ammonia nitrogen and the significant reduction in growth at 0.06 mg/L in the fish early life stages.

4. We recommend that experiments be conducted on the sensitivity of juvenile fish to ammonia at winter temperatures. In this investigation, experiments were run at typical spawning temperatures for the fish species tested. Some information in the literature indicates that fishes may be more sensitive to ammonia at lower temperatures. Thus, it is possible that juveniles exposed to winter temperatures could be a more sensitive life stage than the larval stage.

IV. MATERIALS AND METHODS

TEST SPECIES

Toxic effects of ammonia were evaluated for four fish species and the microcrustacean *Daphnia magna*. Acute lethal toxicity was determined for walleye (*Stizostedion vitreum*) and green sunfish (*Lepomis cyanellus*) larvae and for *D. magna*. Chronic toxic effects were evaluated for *D. magna* and sublethal effects on early life stages of green sunfish, white sucker (*Catostomus commersoni*), and channel catfish (*Ictalurus punctatus*).

Fertilized eggs of walleye and white sucker were obtained from state fish hatcheries and channel catfish eggs from a commercial fish farmer. Green sunfish eggs were artificially fertilized in the laboratory. Sexually mature adults were collected from a population maintained in outdoor ponds at the Illinois Natural History Survey (INHS). No fish were hybrids. Sources of eggs and larval fish are listed in Tables 1 and 2.

Table 1. Source, age of fertilized eggs at exposure, incubation time and temperature, and duration of exposure for species used in the tests on the sublethal effect of ammonia to early life stages.

Species	Source	Age at exposure	Incubation time	Mean temperature (range)	Duration of test
White sucker	Wolf Lake, MI	3 d	7-8 d	18.2 (18.0-19.5)	29 d
Green sunfish	Champaign, IL	<24 h	2 d	26.0 (25.0-27.2)	31 d
Channel catfish	Centralia, IL	<36 h	6-8 d	26.2 (25.2-27.0)	30 d

Larval fish used in acute tests were acclimated in dilution water in the laboratory at the test temperatures for 2-9 d before testing. Twice daily during acclimation, larval fish were fed recently hatched brine shrimp nauplii (*Artemia*) (San Francisco Bay Brand, Metaframe, Inc.).

Table 2. Source and age after hatching of species used in the tests on the acute toxicity of ammonia to larval stages.

Species	Source	Age
Walleye	Shelbyville, IL	4 d
	St. Paul, MN	6 d
Green sunfish	Champaign, IL	9 d

Daphnia magna used in toxicity tests came from cultures maintained in dilution water at 20°C for two generations prior to testing. Cultures were started with daphnids from a culture continuously maintained for more than 5 years at INHS.

DILUTION WATER

Dilution water was taken from municipal wells 220-370 ft deep in the Mahomet-Teays aquifer near Champaign-Urbana. The water was passed through two in-line charcoal filters to remove chlorine, through clinoptilolite when necessary to remove background ammonia, and through an ultraviolet sterilizer to eliminate microorganisms and was then delivered through PVC pipe to a stainless steel holding tank. Sodium thiosulfate was metered into the holding tank to remove any trace of chlorine that might remain after charcoal filtration. Characteristics of the dilution water are listed in Table 3.

EXPOSURE SYSTEMS

Dilution water was supplied from a 670-L stainless steel holding tank. Temperature was controlled by a thermistor in conjunction with two solenoid valves, which allowed hot or cold water to pass through a water jacket surrounding the tank. Water in the tank was aerated.

For each test, a 0.5-L proportional diluter, modified from Mount & Brungs (1967) and Lemke *et al.* (1978), was used to deliver a logarithmic series of five ammonia concentrations and a control through mixing chambers to two replicate test aquaria. Filling of the valve bucket tripped a microswitch which, in conjunction with an electronic timer, controlled a solenoid valve in the water supply line to provide a flow of 0.25 L of water to each test chamber every 6 min. Three diluters were used. The holding tank and diluters were cleaned weekly to prevent bacterial build-up and to maintain a constant pH level.

Reagent-grade ammonium chloride was used as the toxicant. Stock solutions were prepared in distilled water and delivered to diluters from a Mariotte bottle. The pH of the stock solution was adjusted to that of the dilution water with a sodium hydroxide solution.

Test chambers used for fish were constructed of glass and silicone sealant. Each aquarium measured 30 x 20 x 15 cm, had an overflow outlet at a height of 22.5 cm, and contained a volume of 10 L. Test aquaria were placed in a stratified random arrangement and were maintained in circulating water baths at the recommended temperature for the species being tested. Egg incubation cups, made from 4-oz, 5.5-cm OD round glass jars

Table 3. Chemical characteristics of dilution water. All values are in mg/L unless otherwise stated.

Total alkalinity (as $CaCO_3$)	108
Hardness (as $CaCO_3$)	72[a]
Conductivity (µmhos/cm at 25°C)	308
Nitrate-N	0.07
Nitrite-N	0.01
Total ammonia-N	0.01
Soluble orthophosphate	0.01
Total residue	143 [a]
Chloride	11.2
Sulfate	10.7
Total dissolved solids (as NaCl)	230
COD	3.0
Fluoride	1.0
Cyanide	<0.002
Al	<0.201
As	<0.064
Ca	13.2
Cd	<0.005
Co	<0.005
Cr	<0.007
Cu	<0.014
Fe	<0.046
Hg	0.00005
K	2.15
Mn	<0.007
Na	31.42
Ni	<0.015
P	<0.088
Pb	<0.031
Se	<0.042
Zn	<0.021
Residual chlorine	<3

[a]Except during green sunfish early life stages test, when hardness was 54 and total residue 132.

with the bottoms cut off and replaced with 40-mesh nylon screen, were oscillated in the test water using a rocker-arm apparatus driven by a 2-rpm motor (Mount 1968).

Daphnia were exposed in 2-L beakers, two individuals at each of five test concentrations plus controls per proportional diluter, with a flow of 0.125 L of water to each test chamber every 6 min. Flow-splitting cells with capillary tubes were added to prevent sudden pulses of water generated by standard siphon tubes. A chronic renewal test was conducted with *Daphnia* in an environmental chamber with individuals exposed in 200 mL of water in 250-mL beakers, 10 at each of five test concentrations plus control, with test solutions renewed three times each week.

For all flow-through tests, a 16-h photoperiod was maintained, including a 30-min gradual brightening and dimming to simulate dawn and dusk. A combination of incandescent and fluorescent bulbs was used in the laboratory with wide-spectrum fluorescent bulbs (Durotest "Vita Lite") directly over test chambers in fish tests and the daphnia acute toxicity

test. The environmental chamber used in the daphnia chronic test was lighted by cool-white fluorescent bulbs on a 16-h photoperiod.

ANALYTICAL PROCEDURES

Water quality parameters were measured using standard methods (American Public Health Association *et al.* 1976, U.S. Environmental Protection Agency 1979). Water samples were taken from the center of each test chamber.

Total ammonia nitrogen concentrations were determined by the phenate method (American Public Health Association *et al.* 1976) using a standard curve prepared by linear regression. Colorimetric measurements were made with a Coleman 124D double-beam spectrophotometer. Un-ionized ammonia nitrogen (NH_3-N) concentrations were determined from total ammonia nitrogen, pH, and temperature, using the tables of Thurston *et al.* (1979). The pH in each test chamber was determined at least daily with an Orion 701A digital pH meter. Dissolved oxygen was measured with an oxygen-specific electrode calibrated to titration accuracy (Altex 0260 oxygen analyzer by Beckman).

Hardness, nitrate nitrogen, nitrite nitrogen, and soluble orthophosphate were determined using a Technicon Autoanalyzer (U.S. Environmental Protection Agency 1979). Other water quality parameters, such as alkalinity, conductivity, and COD, were determined according to analytical procedures described in American Public Health Association *et al.* (1976). Analyses of metals in the dilution water were performed by induction-coupled argon plasma spectrometry (American Society for Testing and Materials 1980).

TEST PROCEDURES

Acute Toxicity Tests

Methodology for these tests generally followed those in "Methods for acute toxicity tests with fish, macroinvertebrates and amphibians" (U.S. Environmental Protection Agency 1975). The diluter was operated with toxicant introduced into test chambers for at least 24 h before test organisms were added to achieve equilibrium ammonia concentrations in test chambers. Walleye tests were initiated by adding larval fish one at a time to each test chamber. Green sunfish and *Daphnia* were added one at a time to each of 12 beakers containing about 50 mL of dilution water and all organisms from each beaker were then transferred simultaneously into the test chamber. Ages of fish used in acute tests are listed in Table 2. *Daphnia* were less than 24-h old at the initiation of the test.

Larval fish were fed newly hatched brine shrimp twice daily during the test. Daphnids were fed twice daily by adding a sufficient volume of yeast food stock solution to yield 15 mg/L of solids in each test chamber. The food solution was prepared by adding 14.3 g of active dry yeast and 41.8 g of sugar to 0.5 L of dilution water.

Mortality was recorded after 1, 3, 6, 12, and 24 h and at least daily thereafter to the end of the test. Death was determined by lack of a heart beat in fish larvae and by immobility in daphnia.

Tests of Sublethal Effects

Fish. Methodology for these tests generally followed that proposed in "Recommended bioassay procedure for egg and fry stages of freshwater fish" prepared by the National Water Quality Laboratory, Duluth, MN (U.S. Environmental Protection Agency 1972a). In addition, we reviewed drafts of reports of ASTM Committee E-35 on Pesticides, Task Group on conducting toxicity tests with early life stages of fishes.

Egg exposures, except those of channel catfish, were initiated by adding no more than 5 eggs at a time to a total of 100 to each of two egg cups per test chamber. Because channel catfish eggs are in a gelatinous matrix, individual eggs cannot be separated without risk of damaging the chorion. These eggs, therefore, were separated from the mass in groups of approximately 50 each. Four groups (two in each of two egg cups) were placed in each test chamber.

Mortality was recorded daily, and dead eggs were removed to prevent fungus growth. Eggs were determined to be dead when the contents began to turn opaque white. Fry mortality was determined by lack of a heart beat. Tme of hatch and hatching success for each group were recorded. After hatching was completed, surviving white sucker or green sunfish fry were transferred from egg cups into each test chamber. Two weeks after the initiation of the green sunfish test, when fish had been lost from some test chambers, numbers in the remaining test chambers were reduced to 40 each to more nearly equalize the numbers in all chambers. When hatching of channel catfish eggs was completed, 50 fry (25 from each egg cup) were released into each test chamber. Numbers were reduced at that time to prevent crowding and improve growth conditions for larger catfish larvae.

Beginning at the time of swim-up, fry were fed newly hatched brine shrimp twice daily. The test aquaria were siphoned daily to remove fecal material and detritus, and twice each week the sides and bottoms of the chambers were gently brushed to remove bacterial and algal growths.

Specific information on incubation times and test durations are shown in Table 1. All tests were terminated after 29-31 d; surviving fry were individually measured and group weights were determined.

Daphnia magna. Test procedures followed "Recommended bioassay procedure for *Daphnia magna* chronic tests in a flowing system" (U.S. Environmental Protection Agency 1972b) and the proposed standard practices for conducting *D. magna* renewal toxicity tests (Daphnia Task Group, ASTM Committee E-35).

In the chronic renewal test, *Daphnia* were exposed for 21 d in 250-mL beakers, 10 at each concentration. Stock solutions were prepared at five concentrations in a logarithmic series plus a control and the pH of each stock test solution was adjusted to that of the control with a 10-M solution of sodium hydroxide. A food solution was prepared by adding 14.3 g of active dry yeast and 41.8 g of sugar to 0.5 L of dilution water. This solution contained 30 mg/mL of solids. Sufficient volume of this food stock was added to each toxicant stock solution to yield 15 mg/L of solids.

To each of 10 beakers per concentration, 200 mL of stock solution were added. The beakers were separated into two sets: 7 reproduction beakers and 3 survival beakers. One *Daphnia* was added to each reproduction beaker and five individuals were added, one at a time, to each survival beaker. Stock solutions were renewed three times per week.

Mortality and reproduction counts were made three times per week just prior to renewal of the toxicant solution. Young were counted and removed, and original test organisms were transferred into new solutions with a glass pipet. At the end of the test, lengths of surviving first-generation *Daphnia* were measured to the nearest 0.01 mm.

DATA ANALYSIS

Acute Toxicity Tests

Median lethal concentration (LC50) values in the acute toxicity tests were determined using the trimmed Spearman-Karber method (Hamilton *et al.* 1977). When it was necessary to adjust for mortality in the control, Abbott's formula was used (American Public Health Association *et al.* 1976).

Sublethal Effects Tests

One-way analysis of variance (ANOVA) was primarily used in evaluating significance of differences between treatments. Data on group weights, time to swim-up, mortality, and daphnid reproduction and lengths were subjected to ANOVA, and when treatment effects were indicated, were followed by Student-Newman-Keul's or Duncan's New Multiple Range test to determine which treatment effects were significantly different from the controls. For fish length data, the analysis of variance program from the computer package Statistical Package for the Social Sciences (Hine *et al.* 1975) was used, followed by Student-Newman-Keul's multiple range test.

For comparison, swim-up data were also analyzed by 2 x 2 contingency tables. Results were comparable to those from ANOVA. Data on daphnid survival in the chronic test were also analyzed by 2 x 2 contingency tables, using the χ^2 test for goodness of fit.

The maximum concentration causing no effects is defined by Maki (1979) as lying somewhere between the lowest-observed-effect concentration (LOEC) and the highest-observed no-effect concentration (NOEC). NOEC and LOEC values from chronic tests are used to define the range of the estimated maximum no-effect concentration.

V. RESULTS

ACUTE TOXICITY TESTS

Because no data were found in the literature on acute toxicity of ammonia to walleye or green sunfish fry, 96-h acute toxicity tests were conducted with larval walleye from Minnesota and Illinois and green sunfish from Illinois at 6, 4, and 9 d after hatch, respectively (Table 2). The conditions during the tests and results are presented in Table 4.

For walleye, the 96-h LC50 (NH_3-N) values were 0.30 and 0.70 mg/L for fish from Illinois and Minnesota, respectively. The 96-h LC50 for green sunfish was 0.89 mg/L, and the 48-h and 24-h LC50 values for *Daphnia* were 4.07 and 4.72 mg/L NH_3-N, respectively.

Table 4. Acute toxicity (96-h LC50, 95% confidence interval) of ammonia to swim-up fry of walleye and green sunfish and to *Daphnia magna*. Temperature is in °C, dissolved oxygen as % saturation, and un-ionized ammonia and total ammonia in mg/L.

	Temperature Mean (range)	pH range	Dissolved oxygen Mean(range)	96-h LC50 (95% CI) Un-ionized ammonia Mean (range)	Total ammonia Mean (range)
			Walleye		
I[a]	18.3 (17.0-19.6)	8.17-8.61	100	0.30 (0.27-0.33)	3.89 (3.22-4.68)
II[b]	18.2 (17.8-18.7)	7.84-8.31	97 (93-100)	0.70 (0.68-0.72)	13.47(13.12-13.84)
			Green sunfish		
	26.2 (24.4-27.2)	8.46-8.09	88 (78-91)	0.89 (0.81-0.98)	8.93 (8.00-9.98)
			Daphnia magna		
	19.7 (19.4-20.2)	8.58-8.11	95 (90-97)	4.07 (4.07-4.07)	61.3 (61.3-61.3)

[a]Walleye obtained from Illinois
[b]Walleye obtained from Minnesota

SUBLETHAL EFFECTS TESTS

Walleye

Two attempts were made to complete a test on early life stages with walleye from Lake Shelbyville, Illinois. Survival at 2 wk after beginning the test was 20% or less in all test chambers, including controls. Others using walleye eggs obtained from Lake Shelbyville during the same month found similar mortality. The Illinois Department of Conservation had 80% mortality in eggs held to hatch (personal communication, M. Whitacre), and INHS personnel rearing walleye from eggs from the same source had mortalities similar to ours in both embryo and larval stages (personal communication, K. Clement). Others have also reported poor survival (≤30%) of walleye in tests (Sauter *et al.* 1976).

Additional walleye eggs were obtained from the Minnesota State Fish Hatchery in St. Paul, but these eggs hatched in flight to our laboratory. The larval fish were subsequently used in an acute toxicity test, as described earlier.

White Sucker

Conditions during the test are presented in Table 5 and results are shown in Table 6. There were no significant effects on hatching success or larval survival from exposure to NH_3-N concentrations as high as 0.197 mg/L. Time to swim-up was delayed at concentrations of 0.058-0.197 mg/L NH_3-N. Total lengths of sucker larvae exposed to 0.058-0.197 mg/L NH_3-N were significantly less than those of control larvae and of larvae exposed to lower concentrations of ammonia.

Table 5. Conditions during test on sublethal effects of ammonia on early life stages of white sucker. Values for temperature and pH are means of all measurements in all test chambers. Temperature is °C, dissolved oxygen is % saturation, and total and un-ionized ammonia is mg/L.

	Temperature Mean (range)	pH range	Dissolved oxygen Mean(range)	Total ammonia Mean (range)	Un-ionized ammonia Mean (range)
A	18.5 (17.2-20.2)	8.02-8.64	75 (87-58)	control	
B	18.6 (17.2-20.1)	8.03-8.64	73 (85-55)	control	
A	18.7 (17.2-20.1)	8.04-8.56	71 (89-53)	0.39 (0.31-0.48)	0.023 (0.013-0.047)
B	18.8 (17.5-20.3)	8.07-8.65	74 (87-62)	0.29 (0.23-0.34)	0.023 (0.012-0.045)
A	18.8 (17.3-20.3)	8.04-8.63	73 (87-52)	0.53 (0.38-0.68)	0.040 (0.024-0.077)
B	18.5 (17.1-19.9)	8.04-8.63	70 (87-53)	0.50 (0.40-0.64)	0.034 (0.020-0.064)
A	18.5 (17.2-19.9)	8.05-8.62	70 (81-56)	0.97 (0.69-1.18)	0.068 (0.035-0.119)
B	18.9 (17.2-20.5)	8.03-8.62	70 (83-55)	0.85 (0.64-1.32)	0.058 (0.033-0.123)
A	18.7 (17.4-20.1)	8.01-8.60	69 (84-57)	1.50 (1.10-1.89)	0.101 (0.062-0.170)
B	18.5 (16.9-19.8)	8.07-8.63	68 (86-52)	1.48 (1.05-1.95)	0.101 (0.068-0.180)
A	18.6 (17.2-20.1)	8.06-8.58	70 (86-59)	2.88 (2.16-3.55)	0.176 (0.121-0.291)
B	18.6 (17.2-20.1)	8.02-8.60	74 (86-57)	2.88 (2.25-3.65)	0.197 (0.122-0.315)

Table 6. Hatchability, larval survival, development, and growth data for eggs and larvae of white sucker exposed to ammonia for 1 month.

	Mean un-ionized ammonia (mg/L)	% hatch	% survival	% swimming up 72 h after hatch	Mean total length (mm)	Mean weight (mg)
A	Control	96.5	65.4	65.0	15.6	8.7
B	Control	90.5	68.5	77.5	14.8	10.7
A	0.023	93.0	60.8	82.5	14.5	10.2
B	0.023	93.5	62.0	76.9	15.3	12.5
A	0.040	92.5	73.0	67.4	15.3	11.2
B	0.034	85.5	73.7	72.7	14.6	9.4
A	0.068	95.5	65.4	55.6[b]	14.1[a]	14.7
B	0.058	82.0	67.7	64.9[b]	14.7[a]	10.4
A	0.101	94.0	61.7	45.0[b]	14.3[a]	10.8
B	0.101	94.0	68.1	55.0[b]	14.6[a]	11.0
A	0.176	91.5	56.3	40.9[a,b]	14.0[a]	10.5
B	0.197	90.5	64.1	15.8[a,b]	14.4[a]	10.7

[a]Values significantly lower than controls (Student-Newman-Keul's test, $P = 0.05$).

[b]Values significantly lower than controls (2 x 2 contingency table using number swimming up rather than percentage, $P = 0.05$).

Channel Catfish

Test conditions and results are listed in Tables 7 and 8, respectively. No significant effect on hatching success or larval survival was observed during continuous exposure to un-ionized ammonia concentrations as high as 0.480 mg/L. Some mortality was apparently caused by a fungus infection. As observed for white suckers, time to swim-up was delayed at all test concentrations relative to the controls. Continuous exposure to 0.323-0.480 mg/L NH_3-N significantly reduced total lengths and weights of the larval catfish compared with those of controls and of fish exposed to lower treatment concentrations.

Green Sunfish

Test conditions and results for green sunfish are listed in Tables 9 and 10, respectively. Continuous exposure to NH_3-N concentrations as high as 0.96 mg/L had no significant effect on hatching success but larval mortality increased at 0.66 and 0.96 mg/L. Time to swim-up was delayed at 0.96 mg/L, and no fry survived continuous exposure to that concentration for longer than 10 d.

In addition to increased mortality at the two highest test concentrations, some fry were lost from additional chambers. The small size of the fry necessitated the of a fine-meshed screen on overflow tubes in test chambers. Although screens were cleaned 2-3 times/d, they clogged easily, and overnight overflows caused loss of fish from some chambers. As a result of mortality and the overflows, numbers of individuals per chamber were no longer equal. Thus, 2 wk after initiation of the test, numbers of fry in remaining chambers were reduced to approximately 40 each. By the end of the test, due to additional mortality, there were still fewer individuals in both replicates (11 and 21) at the 0.66 mg/L test concentration and in replicate A (26) at 0.23 mg/L compared to other test chambers (36-

Table 7. Conditions during test on sublethal effects of ammonia on early life stages of channel catfish. Values for temperature and pH are means of all measurements in all test chambers. Temperature is °C, dissolved oxygen is % saturation, and total and un-ionized ammonia is mg/L.

	Temperature Mean (range)	pH range	Dissolved oxygen Mean(range)	Total ammonia Mean (range)	Un-ionized ammonia Mean (range)
A	25.9 (24.8-28.3)	7.75-8.37	76 (96-64)	control	
B	26.2 (24.8-28.2)	7.94-8.37	73 (94-62)	control	
A	25.2 (24.8-28.2)	7.57-8.27	73 (91-62)	1.05 (0.45-1.42)	0.059 (0.028-0.095)
B	25.5 (24.8-28.3)	7.73-8.23	74 (93-60)	0.90 (0.58-1.16)	0.050 (0.028-0.074)
A	26.2 (24.8-28.1)	7.65-8.13	75 (94-64)	1.92 (1.47-2.44)	0.116 (0.049-0.192)
B	26.1 (24.8-28.0)	7.65-8.17	74 (91-62)	2.00 (1.36-2.68)	0.115 (0.043-0.206)
A	26.2 (24.9-28.3)	7.57-8.06	73 (90-60)	2.91 (2.48-4.32)	0.169 (0.125-0.270)
B	26.5 (24.9-28.4)	7.53-8.04	70 (91-56)	2.88 (2.36-4.38)	0.157 (0.108-0.248)
A	26.2 (25.0-28.1)	7.68-8.15	75 (91-62)	5.16 (4.70-6.70)	0.323 (0.219-0.450)
B	26.0 (24.9-28.3)	7.66-8.13	74 (93-63)	5.28 (5.10-6.90)	0.338 (0.209-0.510)
A	25.6 (24.9-28.2)	7.56-8.01	72 (94-60)	10.29 (8.64-11.70)	0.453 (0.324-0.604)
B	26.1 (24.9-28.0)	7.57-8.05	73 (94-60)	10.91(10.44-11.88)	0.480 (0.351-0.636)

Table 8. Hatchability, larval survival, development, and growth data for eggs and larvae of channel catfish exposed to ammonia for 1 month.

	Mean un-ionized ammonia (mg/L)	% hatch	% survival	% swimming up 96 h	% swimming up 105 h	Mean total length (mm)	Mean weight (mg)
A	Control	73.0[a]	86.0[a]	100	100	21.9	62.0
B	Control	92.5	100.0	100	100	21.6	60.0
A	0.059	94.5	94.0	64[b,c,d]	84	21.6	61.0
B	0.050	95.5	100.0	62[b,c,d]	80[d]	21.4	60.0
A	0.116	92.0	99.5	52[b,c,d]	70[b,c,d]	21.0	54.0
B	0.115	97.0	98.5	46[b,c,d]	48[b,c,d]	21.7	60.0
A	0.169	78.0[a]	74.0[a]	38[b,c,d]	40[b,c,d]	21.4	61.0
B	0.157	80.5[a]	96.0[a]	68[b,c,d]	68[b,c,d]	21.3	58.0
A	0.323	94.5	97.0	40[b,c,d]	54[b,c,d]	20.4[b]	48.0[c]
B	0.338	98.5	99.0	40[b,c,d]	40[b,c,d]	21.0[b]	54.0[c]
A	0.453	95.0	99.0	2[b,c,d]	8[b,c,d]	20.3[b]	49.0[c]
B	0.480	93.0	99.0	10[b,c,d]	20[b,c,d]	20.9[b]	53.0[c]

[a]Fungus present on egg masses.
[b]Values significantly lower than controls (Student-Newman-Keul's test, $P = 0.05$).
[c]Values significantly lower than controls (Duncan's new multiple range test, $P = 0.05$).
[d]Values significantly lower than controls (2 x 2 contingency table using number-swimming up rather than percentage, $P = 0.05$).

Table 9. Conditions during test on sublethal effects of ammonia on early life stages of green sunfish. Values for temperature and pH are means of all measurements in all test chambers. Temperature is °C, dissolved oxygen is % saturation, and total and un-ionized ammonia is mg/L.

	Temperature Mean (range)	pH range	Dissolved oxygen Mean(range)	Total ammonia Mean (range)	Un-ionized ammonia Mean (range)
A	25.2 (23.8-27.2)	7.92-8.54	87 (100-78)	control	
B	25.4 (23.6-27.0)	7.99-8.56	87 (98-78)	control	
A	25.4 (23.8-27.3)	7.94-8.43	87 (97-75)	1.3 (1.2-1.4)	0.13 (0.10-0.15)
B	25.4 (23.9-27.0)	7.94-8.48	88 (99-78)	1.3 (1.1-1.5)	0.13 (0.10-0.14)
A	25.4 (23.9-26.9)	7.86-8.47	87 (100-77)	2.0 (1.8-2.3)	0.23 (0.20-0.25)
B	25.6 (24.1-27.3)	7.89-8.44	86 (100-67)	2.2 (2.0-2.7)	0.24 (0.21-0.26)
A	25.5 (24.0-27.1)	7.88-8.40	87 (100-77)	3.4 (3.1-3.9)	0.33 (0.27-0.37)
B	25.3 (23.4-27.1)	7.92-8.45	87 (98-69)	3.4 (3.0-3.9)	0.33 (0.26-0.37)
A	25.3 (23.8-26.9)	7.89-8.42	88 (100-70)	6.3 (5.6-7.1)	0.66 (0.61-0.67)
B	25.4 (23.3-27.0)	7.89-8.45	88 (100-72)	6.3 (5.6-7.4)	0.66 (0.65-0.67)
A	25.6 (24.0-27.3)	7.82-8.39	87 (100-70)	9.7(9.2-10.0)	0.96 (0.91-1.0)
B	25.4 (23.9-27.2)	7.82-8.40	85 (100-65)	9.4 (9.0-9.9)	0.95 (0.90-0.99)

Table 10. Hatchability, larval survival, development, and growth data for eggs and larvae of green sunfish exposed to ammonia for 1 month.

	Mean un-ionized ammonia (mg/L)	% hatch	% survival	% swimming up 96 h after hatch	Mean total length (mm)	Mean weight (mg)
A	Control	99	73.0	99	10.9	12.2
B	Control	99	73.5	100	11.0	18.8
A	0.13	99	78.0	98	10.6	10.8
B	0.13	99	87.2	97	11.2	13.1
A	0.23	96	96.0	99	12.2[b]	15.0[b]
B	0.24	98	78.0	98	11.1	11.5
A	0.33	99	77.0	90	10.7	10.9
B	0.33	96	84.2	92	10.9	11.2
A	0.66	96	57.5[a]	96	12.9[b]	19.4[b]
B	0.66	96	44.0[a]	86	11.4[b]	13.6[b]
A	0.96	98	0.0[a]	87[a]		
B	0.95	99	0.0[a]	77[a]		

[a]Values significantly lower than controls (Student-Newman-Keul's test, $P = 0.05$).
[b]Fewer individuals than in other test chambers.

41). In addition to differences in numbers, the small size of the fry made it difficult to distinguish growth effects. For these reasons, no significant effect of ammonia on growth could be identified.

Daphnia magna

A 21-d chronic renewal test was conducted with *Daphnia magna*. Conditions during the test are shown in Table 11. Results of the test as mean values of replicates are presented in Table 12. Survival was slightly less at the two highest test concentrations (73%) than at the other treatments (86-100%), but were not significantly different from the control (2 x 2 contingency table analysis). Daphnid growth, as shown by length, was significantly reduced at the highest test concentration of 1.3 mg/L NH_3-N. Mean lengths in

Table 11. Conditions during the test on the chronic effects of ammonia on *Daphnia magna*. Temperature is in °C and total and un-ionized ammonia in mg/L.

Temperature Mean (range)	pH range	Total ammonia Mean(range)	Un-ionized ammonia Mean (range)
20.3 (20.0-20.8)	7.75-8.16	Control	
20.2 (17.8-20.7)	7.74-8.16	3.99 (2.92-4.58)	0.16 (0.08-0.23)
20.1 (19.8-20.5)	7.70-8.14	6.85 (5.06-7.44)	0.28 (0.14-0.42)
20.1 (19.9-20.6)	7.72-8.12	11.75 (8.30-13.08)	0.47 (0.23-0.73)
20.1 (19.7-20.4)	7.70-8.16	19.66(14.94-23.36)	0.79 (0.38-1.25)
20.1 (19.7-20.5)	7.63-8.16	33.07(25.08-39.06)	1.30 (0.53-2.06)

Table 12. Survival of adults, growth, and reproduction of *Daphnia magna* in a 21-d chronic ammonia toxicity test. Un-ionized ammonia concentrations were measured in mg/L.

Measured un-ionized ammonia	Adult survival (%)	Mean length of surviving adults (mm)	Day when young first produced	Mean number of young/ adult
Control	86	3.52	7	24.6
0.16	91	3.57	7	21.7
0.28	86	3.61	7	22.7
0.47	100	3.73	7	29.0
0.79	73	3.49	7	20.8
1.30	73	3.02[a]	9	6.4[a]

[a]Values significantly lower than controls (Student-Newman-Keul's test, $P = 0.05$).

the control and in the high concentration were 3.52 and 3.02 mm, respectively. Mean lengths at the three lowest treatment concentrations were slightly higher than in the control but were not significantly different.

Reproduction was also affected. The first young were produced on day 7 of the test in all treatments except the highest concentration, where no young were found until day 9. The average number of young/adult was significantly lower at that concentration—6.4 versus 24.6 in the control. Reproduction in the control versus other treatment concentrations was not significantly different.

VI. DISCUSSION

The results of this study indicate that *Daphnia magna* is less sensitive to ammonia than are the fish species tested. The 48-h and 24-h LC50 values of 4.07 and 4.72 mg/L NH_3-N for *D. magna* are in good agreement with the 24-h LC50 of 4.8 mg/L NH_3-N for *Daphnia* sp. reported by Tabata (1962) and the 64-h apparent threshold LC50 for *D. magna*, calculated from Anderson (1948), of 2-3 mg/L NH_3-N at 25°C and a pH range of 8.2-8.4.

Median lethal concentrations of NH_3-N for larval green sunfish and walleye are within the range of toxicity values reported in the literature for other fish species. Roseboom & Richey (1977) reported a 96-h LC50 of 0.4 mg/L NH_3-N for small bluegill with an average weight of 0.072 g at 22°C. For bluegill averaging 0.217-0.646 g at 22-28°C, 96-h LC50 values ranged from 0.49 to 1.3 mg/L. In the pH range similar to that in our tests, Thurston *et al.* (1981) reported 96-h LC50 values for rainbow trout (9.5 g) of 0.51 and 0.66 and for fathead minnows (1.9 g) of 0.65-1.4 mg/L NH_3-N.

The 96-h LC50 for green sunfish fry of 0.81-0.98 mg/L NH_3-N in this test falls within the range of values reported above for other species. Because the green sunfish tested were most comparable in size to the smallest bluegill tested by Roseboom & Richey (1977) and could be more sensitive than larger individuals, green sunfish may be slightly more tolerant of ammonia than are bluegill. On the other hand, Jude (1973) reported an

LC50, presumably at 96 h, of 33 ppm of ammonia as N (approximately 1 mg/L NH_3-N) for green sunfish with an average weight of 8.39 g. This median lethal concentration is only slightly higher than that for the larval green sunfish in our test.

Toxicity values for walleye larvae were lower than for green sunfish, but the 96-h LC50 value of 0.7 mg/L for walleye from Minnesota is within the ranges cited above for bluegill and fathead minnow. Larval walleye from Lake Shelbyville, Illinois, were more than twice as sensitive as those from Minnesota. The apparently greater sensitivity of Illinois walleye to ammonia may be indicative of a more sensitive or less healthy population than those from Minnesota.

There could be several reasons why our early life stages test on walleye was not successful. Generally poor survival rate of Illinois walleye reared in the laboratory could have indicated a problem with the population from Lake Shelbyville. On the other hand, there are problems in working with this species. In their review of biological data on walleye, Colby *et al.* (1979) pointed out that egg viability in walleye may vary widely. Values from 100 to 3.4% have been reported. Others have reported problems in rearing walleye in the laboratory, particularly in feeding them. Sauter *et al.* (1976) reported poor success in feeding walleye fry in toxicity tests on egg and fry stages. Cuff (1977) found that not all walleye in culture learned to feed on brine shrimp, and thus some starved. Cannibalism was also a problem. Such problems need to be addressed to determine whether this species can be successfully used in laboratory tests.

Ammonia concentrations used in fish early life stages tests in this study produced no significant differences in mortalities of fertilized eggs, incubation times to hatching, or percent hatching success for white sucker, channel catfish, or green sunfish. Similar results were reported by Burkhalter & Kaya (1977) for rainbow trout.

NOEC and LOEC values for swim-up and growth for white sucker, channel catfish, and green sunfish in early life stages tests and growth and reproduction of *Daphnia magna* in chronic toxicity tests are listed in Table 13. As in acute toxicity tests, *D. magna* was less sensitive than the fish species tested. The lowest concentration producing an adverse effect in daphnids was 1.3 mg/L NH_3-N while all fish species showed effects at

Table 13. No-observed-effects concentrations (NOEC, mg/L) and lowest-observed-effects concentrations (LOEC, mg/L) for sublethal effects of un-ionized ammonia on white sucker, channel catfish, green sunfish, and *Daphnia magna*..

	Toxicity criterion	NOEC	LOEC
White sucker	time to swim-up	0.040	0.058
	growth	0.040	0.058
Channel catfish	time to swim-up	control	0.050
	growth	0.169	0.323
Green sunfish	time to swim-up	0.66	0.96
Daphnia magna	growth	0.79	1.30
	number of young produced	0.79	1.30

lower concentrations. Both growth and reproduction were affected in daphnids. Since reproduction in daphnids is correlated with body size, however, reduction in the number of young produced may be related to reduction in growth.

In all three fish early life stages tests, time to swim-up was delayed at some treatment concentration. LOEC concentrations were 0.058, 0.050, and 0.96 mg/L NH_3-N for white sucker, channel catfish, and green sunfish, respectively. As shown by the results of counts, at two different times in the case of catfish, the actual percentage of fry that reached swim-up stage depended on the time when the count was made. Differences observed, however, were statistically significant.

Growth reduction in white suckers occurred at concentrations as low as 0.058 mg/L NH_3-N. This is comparable to the level of 0.05 mg/L that Burkhalter & Kaya (1977) found to cause retardation of growth and development in rainbow trout fry.

For channel catfish, the lowest concentration found to decrease growth significantly was 0.323 mg/L NH_3-N, with no significant effect found at 0.169 mg/L. We have found no other information in the literature on effects of ammonia on channel catfish early life stages. For larger individuals (20.3 g) of the same species, Colt & Tchobanoglous (1978) found a 50% reduction in growth during a 31-d trial at 0.517 mg/L NH_3-N, no growth at 0.967 mg/L, a 23% reduction in growth at 0.217 mg/L, and a difference in growth compared with the control at 0.048 mg/L. The lowest value causing an effect on growth in that study is similar to the lowest concentration (0.05 mg/L) found to affect time to swim-up in this investigation.

In summary, results of this investigation indicate that concentrations of NH_3-N as low as 0.05 and 0.06 mg/L caused a delay in time to swim-up and a significant reduction in growth, respectively, in early life stages tests with these fish species.

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Appendix A. ANOVA table and results of Student-Newman-Keul's test for white sucker lengths from early life stages test

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Variable length by treatment

Source	d.f.	Sum of squares	Mean square	F ratio	F probability
Between groups	5	90.012	18.002	14.769	0
Within groups	819	998.335	1.219		
Total	824	1088.348			

Variable length
Multiple range test

Student-Newman-Keul's procedure
Ranges for the 0.050 level

2.81	3.33	3.65	3.87	4.04

The ranges above are tabular values.
The value actually compared with mean (j) - mean (i) is
0.7807 x range x sqrt (1/n(i) + 1/n(j))

Homogeneous subsets (subsets of groups, whose highest and lowest means do not differ by more than the shortest significant range for a subset of that size).

Subset 1			
Group	6	4	5
Mean	14.2055	14.3514	14.4893

Subset 2		
Group	5	2
Mean	14.4893	14.7488

Subset 3		
Group	2	3
Mean	14.7488	14.9154

Subset 4		
Group	3	1
Mean	14.9154	15.1449

Appendix B. ANOVA table and results of Student-Newman-Keul's test for channel catfish lengths from early life stages tests

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Variable length by treatment

Source	d.f.	Sum of squares	Mean square	F ratio	F probability
Between groups	5	103.617	20.723	16.871	0.7
Within groups	586	722.832	1.234		
Total	591	826.449			

Variable length
Multiple range test

Student-Newman-Keul's procedure
Ranges for the 0.050 level
2.81 3.33 3.65 3.83 4.05

The ranges above are tabular values.
The value actually compared with mean (j) - mean (i) is
0.7853 x range x sqrt (1/n(i) +1/n(j))

Homogeneous subsets (subsets of groups, whose highest and lowest means do not differ by more than the shortest significant range for a subset of that size).

Subset 1		
Group	6	5
Mean	20.6010	20.7163

Subset 2				
Group	4	3	2	1
Mean	21.3525	21.3554	21.4960	21.7544
